

REMARKS

Claims 1-35, 37-53, 59-61, 63-66 and 78-86 were previously pending. In the Action, the Examiner has indicated that claims 59-61, 63-66 and 78-86 are allowed and claims 3, 4, 6, 8, 10-17, 19-27, 31, 34, 35, 37-40, 43-45 and 47-53 are not rejected, but are objected to for depending from a rejected claim(s). Claims 60, 61, 63, 64, 78-86 and 105-171 are now pending. The following describes the relationship between new claims 105-171 and formerly pending claims. No new matter is added by this Amendment.

Allowed claim 59 is hereby re-written as claims 170 and 171, which present essentially the same, presumably allowable, subject matter in two claims. Claim 170 is believed to be allowable in view of the subject matter of original claims that do not stand rejected, but were objected to for depending from a rejected claim(s).

Allowed claim 65 is presented as new claim 116, along with claims dependent therefrom. Claim 65, along with other pending claims have been amended to replace the superfluous language "comprising *the step(s) of*" with "comprising".

Independent claim 105, along with claims 106-108, dependent therefrom, combine the matter of Original claim 1 with the matter of "allowable" claims 3, 4, 6 and 11 (presumed to be allowable because they were not rejected) – the transcript/amplicon-specific embodiments.

Independent claim 109, along with claims 110 and 111, dependent therefrom, combine the matter of Original claim 1 with the matter of "allowable" claims 21-23 – the fluorescent reporter quantification claims.

Independent claim 112, along with claim 113, dependent therefrom, combines the matter of Original claim 1 with the matter of "allowable" claims 12 and 13 – the rapid denaturation claims.

Independent claim 114 combines the matter of Original claim 1 with the matter of "allowable" claim 8, in which the annealing and elongation steps are conducted at the same temperature.

Independent claim 115 combines the matter of Original claim 1 with the matter of "allowable" claim 38, in which the annealing temperature is decreased from the first stage to the second.

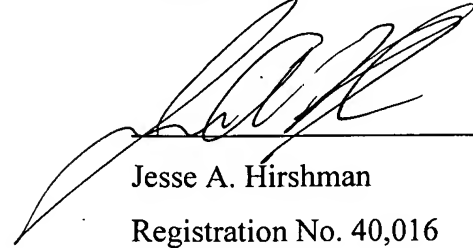
The Examiner has rejected claims 1, 2, 7, 9, 14, 18, 30, 32, 33, 41, 42 and 46 and claims 1 and 28 under 35 U.S.C. §102(b) for anticipation by Howell *et al.* (US 5,985,552) and Durmaz *et al.* respectfully. In response, Applicants hereby present new claims 119-146 and 147-170 which omit the “nested PCR” configuration described in the relevant portions of Howell *et al.* and Durmaz *et al.* These references describe the situation where a first primer pair amplifies a target DNA sequence to produce a first amplicon and a second (or third) primer pair amplifies sequences present in the first amplicon. This is wholly different from the purpose of the presently-described, and claimed, methods, to balance a multiplexed reaction by amplifying different target DNA strands (for example, produced by reverse-transcribing two different mRNA transcripts). In the reactions described in the cited art, the second reaction amplifies an amplicon, not a separate target DNA (*e.g.*, a target cDNA). The kinetics of amplification of two independent target sequences is different from the kinetics of amplification of a “nested” pair, as is described in the cited art. It is not possible to use nested PCR to quantify target sequences, at least as is described in the cited art.

In response, Applicants have amended original claims 1 and 30, now presented as new claims 119 and 147, to exclude the case where the second primer amplifies the amplicon product of the first stage reaction. Independent claims 119 and 147, and thus claims dependent therefrom, now better define over the “nested PCR” examples described in Howell *et al.* and Durmaz *et al.* In light of these amendments, Applicants respectfully request withdrawal of the rejection of claims 1 and 30 (now claims 119 and 147).

Applicant believes that claims 60, 61, 63, 64, 78-86 and 105-171 define over the prior art of record and are in proper form for allowance. Applicant respectfully requests allowance of claims 60, 61, 63, 64, 78-86 and 105-171. Applicant also requests that the Examiner call the

undersigned to discuss any additional questions or concerns with respect to the above-referenced patent application.

Respectfully submitted,



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